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LARVAL AND METAMORPHIC MORPHOGENESIS IN THE NUDIBRANCH *MELIBE LEONINA* (MOLLUSCA: OPISTHOBRANCHIA)

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Abstract

Larval development and metamorphosis in the nudibranch *Melibe leonina* (Gould) are described from observations of living animals and from one micrometer histological sections. Larval morphogenesis is similar to that previously described for other species of planktotrophic opisthobranch larvae except the rudiments of the primary cerata and the oral hood of the post-metamorphic stage appear in the late stage larva. Unlike many other opisthobranch larvae, *M. leonina* does not appear to require a specific exogenous cue to induce metamorphosis. Metamorphosis involves loss of the shell, operculum, velar ciliated cells, and certain components of the larval stomach but the left and right digestive diverticula are retained. A rapid expansion of the primary cerata and the oral hood occurs and is accompanied by a large volume increase of the internal hemocoel of these structures and a flattening and vesiculation of their epithelial cells. Several neuronal somata within the pleural ganglia become notably larger than their neighbors during metamorphosis. At approximately 2.5 days after shell loss, *M. leonina* begins to employ the oral hood to capture ciliates and small benthic nauplii. Morphogenesis in *M. leonina* is compared to that of other opisthobranchs and the premetamorphic appearance of the cerata and the lack of an exogenous metamorphic trigger are discussed.

Introduction

A number of histological and ultrastructural studies of opisthobranch morphogenesis during the larval, metamorphic, and juvenile stages have been published during the last 25 years (Thompson, 1958; 1962; Tardy, 1970; Thiriot-Quiévreux, 1970; 1977; Bonar and Hadfield, 1974; Bonar, 1976; Kriegstein, 1977a, b; Bickell, 1978; Bickell and Chia, 1979; Schacher *et al.*, 1979a, b; Bickell *et al.*, 1981; Kempf, 1982). Considered together, these works have elucidated a general pattern of development through metamorphosis in opisthobranchs having a free-swimming larval stage. However, in a comparative sense, the studies have also pointed out specific morphogenetic differences in the ontogeny of larval opisthobranchs with different taxonomic affinities. These include interspecific differences regarding the body size of the metamorphically competent larva, the derivation of the adult dorsal epidermis, the presence or absence of true detorsion of the gut, and the occurrence of characteristics such as the right digestive diverticulum and the rudiments of various adult structures (Bonar, 1978a review; Chia and Koss, 1978; Switzer-Dunlap, 1978; Bickell and Chia, 1979; Kempf, 1982).

The information that has accumulated on opisthobranch morphogenesis suggests several important objectives for future research. These are: 1) further histological and ultrastructural investigations on larval development and metamorphosis to

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distinguish tissue and organ homologies between adults, thereby helping to solve taxonomic questions within the subclass, 2) clarification of the phenomenon of metamorphic induction and its ecological consequences, and 3) individual and comparative studies to examine neurodevelopment in both a morphological and behavioral sense.

Melibe leonina is a large dendronotid nudibranch that often reaches high population densities within eel grass and kelp beds along the west coast of North America (Agersborg, 1923a; Hurst, 1968; Ajeska and Nybakken, 1976). Like certain other members of the Dendronotacea, *M. leonina* exhibits a swimming behavior consisting of rhythmical bending movements of the body. The most distinctive characteristic of this species is the oral hood; a large, highly mobile expansion of cephalic tissue that extends over and around the mouth and bears a double row of inner and outer tentacles along its peripheral edge (Agersborg, 1923b) (Fig. 1). This oral hood expands and contracts through the action of muscles and pumped hemal fluids (Hurst, 1968) and is used to capture the small zooplanktonic organisms that comprise the prey of adult *M. leonina* (Agersborg, 1923a; Ajeska and Nybakken, 1976). Various organisms, notably crustaceans, are engulfed by the hood and subsequent cooperative actions of the hood and oral lips forces the prey into the mouth (Hurst, 1968). This novel method of prey capture is correlated with the absence of a radula in this species (Agersborg, 1923b).

The following study of morphological development during the larval and metamorphic stages of *M. leonina* was undertaken to provide information on larval and metamorphic morphogenesis for comparison with other opisthobranch species, to examine metamorphic induction and survival strategies in a nudibranch that feeds relatively non-specifically during the juvenile and adult stages, and to investigate the potential of *M. leonina* as a system for studying opisthobranch neurodevelopment.

Materials and Methods

Adult *M. leonina* and their egg masses were collected from a number of eel grass and kelp beds located around the San Juan Archipelago (Washington, U. S. A.) and the southern end of Vancouver Island (British Columbia, Canada).

Laboratory hatched larvae were cultured at an initial density of 2 to 3 larvae/ml in bowls containing 100 ml of filtered (Millipore prefilter no. AP2004700) natural sea water with 10^4 cells/ml of the alga *Pavlova (Monochrysis) lutheri* (Carolina Biological Supply). The larvae were transferred to fresh culture medium at 1 or 2 day intervals and the antibiotics streptomycin sulfate (50 μm/ml) and penicillin G (60 μm/ml) (Switzer-Dunlap and Hadfield, 1977) were added at 2 to 6 day intervals. Cultures were maintained at a temperature of 12 to 14°C.

Young juveniles of *M. leonina* were fed a mixture of unidentified ciliates harvested from various types of decomposing animal tissue (sea urchin eggs, crushed limpets, chunks of sea pen). This diet was supplemented with nauplii of harpacticoid copepods.

Ten developmental stages were processed for histological examination. Larvae were fixed at hatching, mantle fold retraction, onset of mantle fold hypertrophy, and full development of the propodium. Metamorphic stages were fixed at the time of velum loss, at shell loss, and at 5, 10, 24, and 48 hours after loss of the larval shell. Primary fixation was accomplished in 2.5% glutaraldehyde and post-fixation in 2% osmium tetroxide as described previously (Bickell and Chia, 1979). Larval stages were anaesthetized prior to fixation by placing them in an incubation vessel containing 3 ml of sea water and 7 drops of 2% procaine. After 15 min at room

temperature, 0.5 ml of a saturated solution of chlorobutanol in sea water was added and the incubation vessel placed on ice for 10 min. Anaesthetized animals were placed in primary fixative for 30 min, followed by a 1 h treatment in a mixture of equal parts primary fixative and 10% ethylenediaminotetraacetic acid (disodium salt) to decalcify the larval shells (Bonar and Hadfield, 1974). Metamorphic stages were anaesthetized for 5 min in 1 part saturated chlorobutanol solution and 9 parts filtered sea water on ice and transferred to primary fixative for 1 h. All larval and metamorphic stages were post-fixed for 1 h. Fixed animals were dehydrated in ethanol and embedded in a plastic prepared by substituting Poly/Bed 812 (Polysciences) for Epon 812 in the recipe of Luft (1961). Embedded specimens were serially sectioned at 1 micrometer thickness and stained with Richardson's stain (Richardson *et al.*, 1960).

Results

Structure of the larva at hatching

The veliger larvae of *Melibe leonina* hatch from the benthic egg mass approximately 10 days after oviposition and are structurally similar to the young planktotrophic veligers of other opisthobranchs. At hatching, the larval body is small and morphologically simple relative to the size and complexity that is achieved by the end of the obligatory larval stage (compare Figs. 2 and 3).

The veliger has two major body regions: a cephalopedal mass and a visceropallial mass. The cephalopedal mass consists of the two ciliated lobes of the velum that effect swimming and capture of food particles, and a small pointed foot that bears a circular operculum on its posterior face (Figs. 2, 4). A ciliary tract extends down the midventral surface of the foot and transports rejected particles away from the mouth.

The visceropallial mass includes a functional digestive tract, the so-called larval kidney complex, and the larval shell with its underlying perivisceral epithelium (Figs. 2, 4). The digestive tract is composed of an esophagus, a stomach, a large left and much smaller right digestive diverticulum, and an intestine (Figs. 2, 4, 5). The intestine leaves the postero-dorsal region of the stomach and recurves anteriorly to terminate at the anus located on the floor of the right mantle cavity (Fig. 5). The larval stomach has two major divisions that Thompson (1959) termed the ventral and dorsal stomach. The ventral stomach consists of a ciliated region that receives the openings of the esophagus and digestive diverticula and an area lined by a gastric shield (Fig. 4). The dorsal stomach is lined on three sides by a band of densely packed, transversely beating cilia (Fig. 6). A sparsely ciliated groove extends down the upper wall of the dorsal stomach (Fig. 6). The band of densely-packed cilia and the sparsely ciliated groove are structurally similar to the style sac ciliation and intestinal groove, respectively, of lamellibranch and some prosobranch molluscs (Graham, 1941). The manner in which food particles are transported and digested by the gut of opisthobranch veligers has been described previously (Thompson, 1959; Bickell *et al.*, 1981; Kempf, 1982). The anterior deflection of the intestine is evidence of partial torsion of the larval digestive tract.

The larval kidney complex is a cluster of distinctive yet heterogenous cells located adjacent to the anus on the right side of the veliger (Figs. 2, 4). The function of these cells, which degenerate at metamorphosis, is not clear. The two nephrocysts are located on either side of the esophagus (Fig. 6). They are uniquely larval structures whose function is enigmatic but may involve storage or excretion of waste material (Bonar, 1978a).

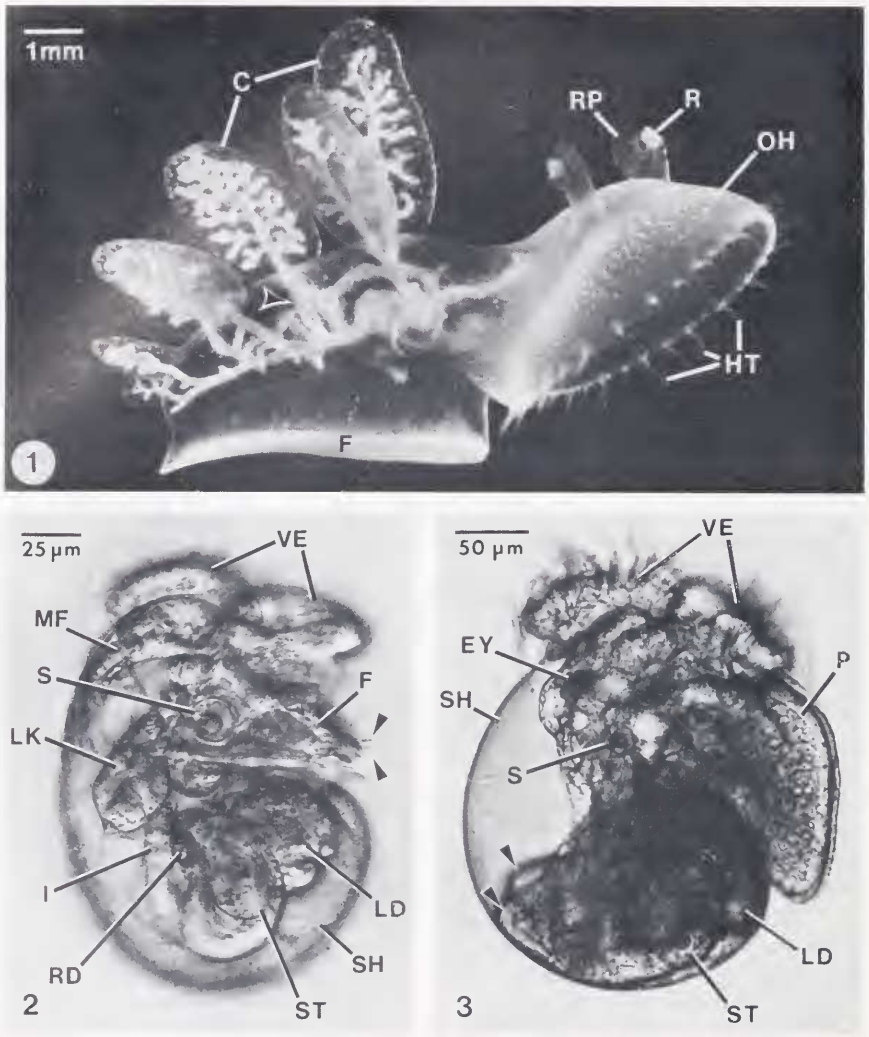


FIGURE 1. Juvenile of *Melibe leonina* at 2.5 months after metamorphosis showing the foot (f), double row of lobate cerata (C) containing dendritic branches of the digestive diverticula, and oral hood (OH) surrounding the mouth. The oral hood bears peripheral hood tentacles (HT) and a pair of rhinophores (R) mounted on a rhinophoral process (RP). The arrowhead indicates the position of the anus.

FIGURE 2. Larva of *M. leonina* immediately after hatching showing the velum (VE), foot (F), and statocyst (S) of the cephalopedal mass and the stomach (ST), right and left digestive diverticula (RD and LD, respectively), intestine (I), larval kidney complex (LK), mantle fold (MF), and shell (SH) of the visceropallial mass. The arrowheads indicate the tuft of long, stiff cilia at the apex of the foot.

FIGURE 3. Late stage larva of *M. leonina* showing the right eye (EY), propodium (P), enlarged stomach (ST), left digestive diverticulum (LD), and the shell (SH), statocyst (S), and velum (VE). The arrowheads indicate the rudiments of the primary cerata.

At the aperture of the shell, the associated perivisceral epithelium is termed the mantle fold and its cells are specialized for secretion of shell material (Fig. 7). The remainder of the mantle extends from the aperture of the shell to the cephalopedal mass and thus demarcates a shallow mantle cavity in the newly hatched veliger.

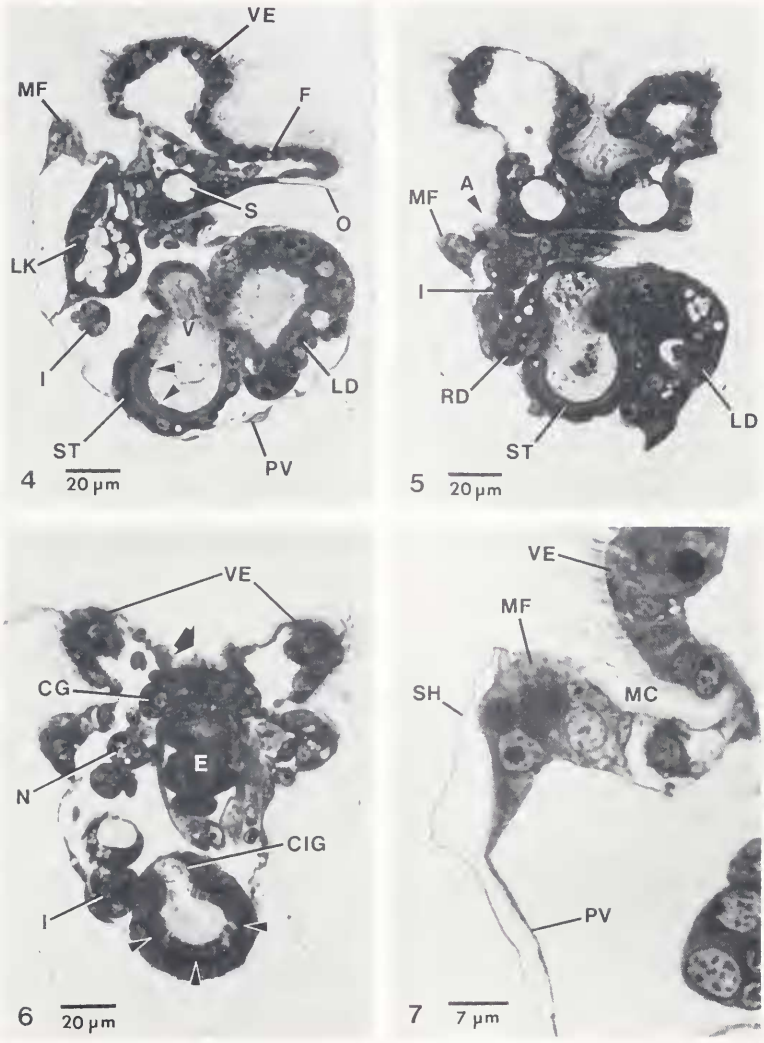


FIGURE 4. Oblique sagittal section of a newly hatched larva of *M. leonina* that passes through the foot (F), operculum (O), statocyst (S), and velum (VE) of the cephalopedal mass and the stomach (ST), left digestive diverticulum (LD), intestine (I), larval kidney complex (LK), perivisceral epithelium (PV), and mantle fold (MF) of the visceropallial mass. The section shows the vestibule (V) and gastric shield (arrowheads) of the larval stomach.

FIGURE 5. Frontal section through a newly hatched larva showing the small right and large left digestive diverticula (RD and LD, respectively) flanking the stomach (ST). Also note the torted intestine (I) that terminates at the anus (A) in the mantle cavity on the right side. Mantle fold (MF).

FIGURE 6. Frontal section through a newly hatched larva showing the sparsely ciliated groove (CIG) and band of dense cilia (arrowheads) within the dorsal part of the larval stomach (style sac). The cerebral ganglia (CG) are connected over the esophagus (E) by a commissure and an apical tuft of cilia (arrow) arises from the cephalic epithelium between the velar lobes (VE). Also note the nephrocyst (N) and intestine (I).

FIGURE 7. Detail of the mantle fold on the right side of a newly-hatched larva. The mantle fold (MF) is a continuation of the perivisceral epithelium (PV) and elaborates shell material (SH) at the shell aperture. A shallow mantle cavity (MC) is demarcated by the mantle and velar (VE) epithelia.

The muscle systems of the veliger of *M. leonina* extend through both the cephalopedal and visceropallial portions of the larval body. The base of the large larval retractor muscle is attached to the posterior end of the shell via specialized cells of the perivisceral epithelium (Bonar, 1978b) and branches extend anteriorly into the tissues of the foot and velum. A bundle of accessory pedal retractor muscles originates on the pedal epithelium underlying the operculum and extends over the ventral lip of the shell to insert on the perivisceral epithelium immediately ventral to the anus. Contraction of the larval retractor and accessory pedal retractor muscles pulls the larval body and operculum into the shell cavity. In addition, a diffuse system of slender visceral muscles are associated with the digestive tract and with the mantle fold and perivisceral epithelium.

At the time of larval hatching in *M. leonina,* the only central ganglia that are clearly recognizable in one micrometer sections are a pair of small cerebral ganglia; these are connected dorsally over the esophagus by the cerebral commissure (Fig. 6). Sensory structures include a pair of statocysts within the base of the foot (Figs. 2, 4), a tuft of stiff cilia extending from the apex of the foot (Fig. 2), and an apical organ that bears a long tuft of cilia and is located within the cephalic epidermis overlying the cerebral commissure (Fig. 6). Bonar (1978c) described the ultrastructure of the apical organ in larvae of the nudibranch *Phestilla sibogae* and suggested that it may be chemosensory.

Larval morphogenesis

The sketches in Figure 8 portray three stages of the larval development of *Melibe leonina:* the hatching stage, the eyespot—mantle retraction stage (16 to 20 days post-hatching), and the stage at which the larvae become capable of settlement and metamorphosis (30 to 48 days post-hatching). The shell increases in length from 149 μm (S.D. 9 μm) at hatching to 250 μm (S.D. 3 μm) (Fig. 8). As described below, morphogenetic events occur throughout the larval phase but tend to be concentrated within the latter half of development.

The mantle fold of *M. leonina* veligers undergoes a series of major morphogenetic changes during the larval stage. After secreting shell material during the initial portion of larval development, the mantle fold epithelium detaches from the rim of the shell and is pulled posteriorly (Figs. 8b, 9), presumably by slender muscles that extend from the mantle fold and larval kidney complex to various sites on the viscera and perivisceral epithelium. The cells of the retracted mantle fold epithelium subsequently proliferate and hypertrophy and cells of unknown origin accumulate along the hemal side of the retracted epithelium. Eventually, the mantle fold becomes composed of closely packed columnar cells and assumes the form of two large protuberances projecting from the postero-dorsal surface of the visceral mass (Figs. 3, 8c, 10). These structures are the rudiments of the primary cerata of the juvenile–adult stage. Hypertrophied mantle fold cells also extend a short distance over the latero-dorsal side of the large left digestive diverticulum and along the right side towards the anus.

The foot of the larva is enlarged considerably by proliferation of the pedal epithelial cells (compare Figs. 4, 9, 10). During the latter half of development, foot growth is accompanied by the differentiation of intrinsic pedal muscles and of large pedal glands that expand within the pedal hemocoel as they become filled with secretory product. These events ultimately result in the development of the propodium, a large swelling on the proximal, ventral surface of the foot (Figs. 3, 10). The full development of the propodium and the concurrent growth of a dense

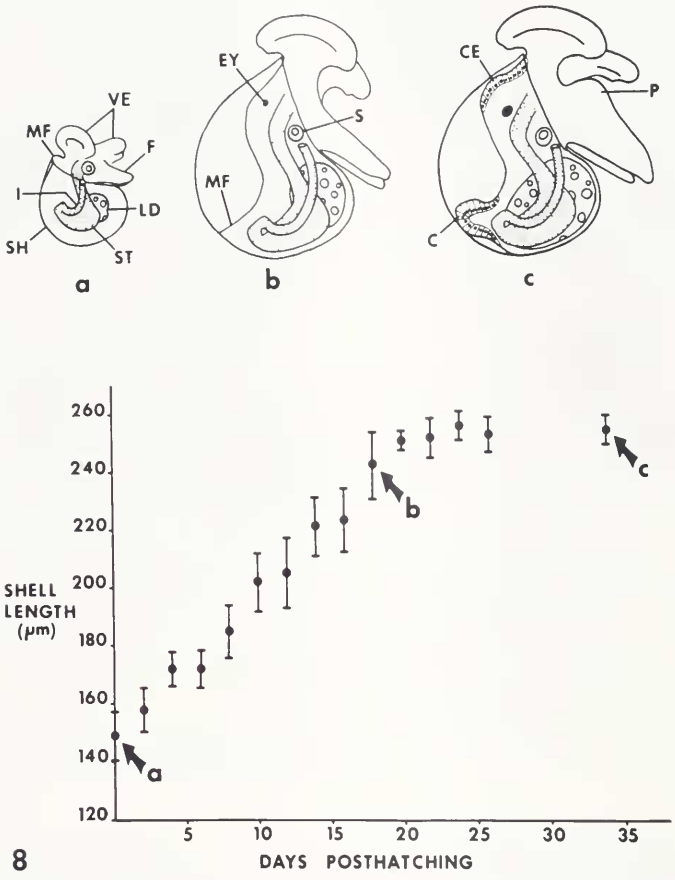
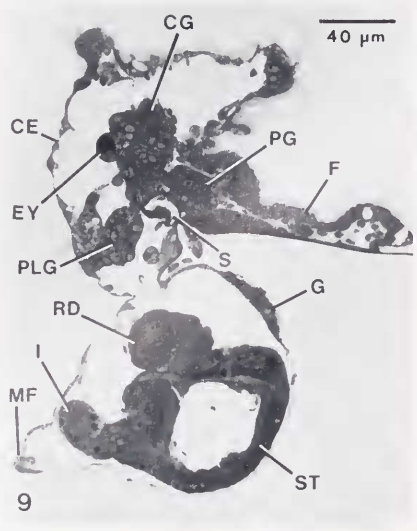


FIGURE 8. Growth rate of the shell during the larval development of *M. leonina*. The points indicate the mean length of a minimum of five larvae and the vertical bars show the standard deviation. Diagrams of the newly hatched stage (a), the mantle retraction stage (b), and the late larval stage (c) correspond in approximate size and age to the sites indicated by arrows on the graph. Abbreviations: C, ceras; CE, hypertrophied cephalic epithelium; EY, eye; F, foot; I, intestine; LD, left digestive diverticulum; MF, mantle fold; P, propodium; S, statocyst; SH, shell; ST, stomach; VE, velum.

covering of cilia over the ventral surface of the foot enables crawling behavior; a phenomenon that provides a convenient marker for the recognition of metamorphically competent opisthobranch veligers.

The cephalic epithelium that lies immediately dorsal and lateral to the velar lobes also exhibits proliferation and hypertrophy during the latter part of the larval development of *M. leonina* (compare Figs. 9, 10). This band of columnar cephalic epithelium will form the epidermis of the post-metamorphic oral hood.

The basic structure of the gut is preserved throughout the larval phase, although the digestive tract grows considerably and the cells of the stomach and left digestive diverticulum accumulate lipid deposits (Fig. 10). In late stage larvae, a vestigial radular rudiment becomes evident as a slight evagination of the ventral wall of the distal esophagus (Fig. 10), but neither radular teeth nor muscles differentiate in association with this outpocketing as typically occurs during the development of other opisthobranch larvae (Bonar, 1978a).



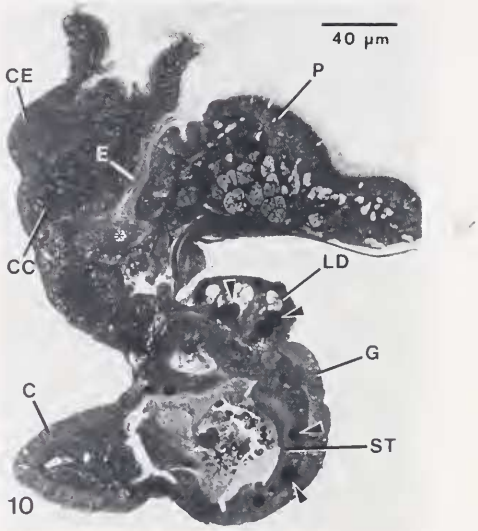


FIGURE 9. Sagittal section through a larva of *M. leonina* in which the mantle fold (MF) has retracted from the aperture of the shell. Note the eye (EY), statocyst (S), cerebral ganglion (CG), pedal ganglion (PG), and pleural ganglion (PLG) of the larval nervous system and the thin cephalic epithelium (CE), the elongate but low profile of the foot (F), and the gonadal rudiment (G). The section also passes through the stomach (ST), right digestive diverticulum (RD), and the intestine (I).

FIGURE 10. Mid-sagittal section through a larva of *M. leonina* just prior to the onset of metamorphosis showing the hypertrophied cephalic epithelium (CE), a ceratal rudiment (C), the propodial swelling (P) on the ventral surface of the foot, the gonadal rudiment (G), and the many large lipid deposits (arrowheads) within the walls of the stomach (ST) and left digestive diverticulum (LD). A vestigial radular rudiment (asterisk) has evaginated from the ventral wall of the esophagus (E) at the level of the cerebral commissure (CC).

The nervous system of *M. leonina* becomes extensively elaborated during larval development. By the time of mantle retraction, the pedal and pleural ganglia are clearly recognizable, the cerebral ganglia have enlarged, and a pair of eyespots have differentiated (Fig. 9). The pedal ganglia differentiate adjacent to the statocysts and are connected to each other by a pedal commissure and to their respective ipsilateral cerebral ganglion by a cerebropedal connective. Each pleural ganglion extends from the ipsilateral cerebral ganglion via a broad cerebropleural connective. Between the stages of mantle retraction and the onset of metamorphosis, the buccal and rhinophoral ganglia differentiate.

Three additional developments that occur during the larval phase of *M. leonina* are the development of the pulsatile larval heart soon after mantle retraction, the appearance of the adult kidney rudiment adjacent to the larval kidney complex and intestine, and the enlargement of the rudiment of the gonad (Fig. 10).

Metamorphosis

Larvae of *Melibe leonina* do not appear to require a specific, external chemical cue for the induction of metamorphosis. After full development of the propodium, the larvae of this species settle onto the foot, exhibit a brief period of crawling, and commence metamorphosis.

The events of metamorphosis that are seen during external inspection of this process are shown in Figures 11 through 16. The first superficial indication that

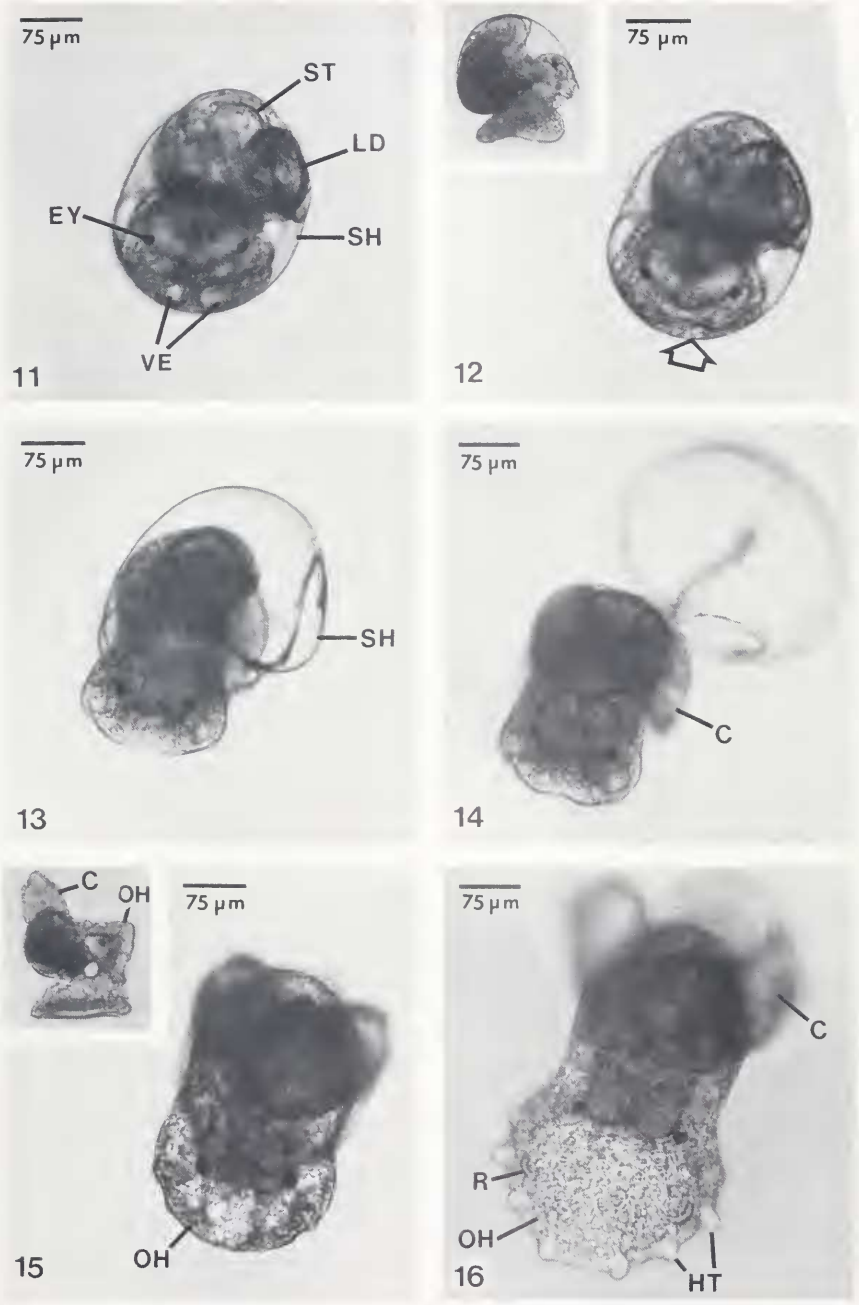


FIGURE 11. Dorsal view of a larva of *M. leonina* that has settled onto the foot in preparation for metamorphosis. The stomach (ST), left digestive diverticulum (LD) and eyes (EY) are visible through the transparent larval shell (SH). The velar lobes (VE) are retracted but still intact.

FIGURE 12. Onset of metamorphosis. The slurry of cells indicated by the arrow are dissociated velar cells. Inset: lateral view of a post-larva after loss of the ciliated velar cells.

FIGURE 13. Post-larva withdrawing the visceral mass from the shell (SH).

FIGURE 14. Post-larva immediately after shell loss. Note the left ceras (C).

FIGURE 15. Post-larva at 10 hours after shell loss showing the initial expansion of the cephalic epithelium to form the oral hood (OH). Inset: lateral view of a post-larva showing a ceras (C) and the oral hood (OH).

FIGURE 16. Post-larva at approximately 36 hours after shell loss showing the cerata (C), the dramatic enlargement of the oral hood (OH), and the buds of the initial hood tentacles (HT). The developing rhinophores (R) appear as two crescent-shaped ridges on the dorsal surface of the oral hood.

metamorphosis is irreversibly underway is the dissociation of the ciliated velar cells (Fig. 12). Many of these cells are ingested but some escape into the surrounding environment. Dissociation of the velar cells is followed by the loss of the operculum and the larval shell (Figs. 13, 14). The time interval between settlement and shell loss is variable but is usually between 12 and 24 hours. During and following shell loss, the cerata and the oral hood undergo a period of rapid and pronounced enlargement (Figs. 15, 16).

Serial sections of *M. leonina* fixed at various stages after settlement reveal that much structural reorganization and tissue morphogenesis occurs during metamorphosis. Some of these changes are illustrated schematically in Figure 17.

Beginning soon after the dissociation of the velar cells, the trunk of the larval retractor muscle becomes detached from the posterior wall of the shell. Subsequent contractions of this muscle appear to pull the visceral mass out of the shell in a manner similar to that described for other nudibranchs (Bonar and Hadfield, 1974; Bonar 1976; Bickell *et al.*, 1981). The larval retractor and accessory pedal retractor muscles degenerate following shell loss.

During and immediately following shell loss, the hypertrophied mantle fold epithelium spreads posteriorly and laterally over the stomach and digestive diverticula and anteriorly toward the hypertrophied epithelium of the presumptive oral hood (Figs. 17a–c). As the migrating edge of the mantle fold epithelium reaches the gonadal rudiment, the latter tissue invaginates and the converging margin of the spreading mantle tissue eventually fuses over the site of this internalization at the posterior extremity of the visceral mass (Figs. 17b, 18). The fate of the perivisceral epithelium is not apparent; it may be sloughed into the environment or overgrown and subsequently phagocytized. Nevertheless, the lateral and posterior margins of the mantle fold epithelium are continuous with the pedal epithelium by 5 hours after shell loss.

The loss of the shell and operculum at metamorphosis permits a broadening of the connection between the visceral mass and the foot (Figs. 17d–g). In *M. leonina*, this process appears to be facilitated by a large increase in the volume of the hemal space within the foot and surrounding the viscera. Inspection of living animals and histological sections of metamorphosing *M. leonina* give the impression that a large volume of external fluid has been pumped through the body wall and into the hemolymph. A similar but much more pronounced expansion of the hemal space accompanies the rapid enlargement of the cerata (compare Figs. 19 and 20) and the oral hood during and following shell loss. As these structures expand, the surface area of their covering epithelia is increased by conversion from a columnar to squamous epithelial type. A marked increase in the vesiculation of the epithelial cells occurs concurrently with their shape change (Figs. 19, 20, 21). Each ceras contains longitudinal muscle fibres and tufts of stiff cilia are distributed along the length of these structures (Figs. 20, 21).

The anus of *M. leonina* is displaced posteriorly following shell loss, presumably by the posterior migration of the mantle fold epithelium that surrounds the anus and by the broadening of the connection between the foot and visceral mass (Figs. 17a, b). Subsequently, the anus moves dorsally along the postero-lateral side of the post-larva. The latter movement appears to be effected by a dorsal shifting of mantle epithelium resulting from the inflation of the cerata and from a dorsally directed spread of pedal epithelium (Fig. 17c). Although the anus is moved posteriorly and dorsally, its definitive location is slightly to the right of the mid-sagittal plane of the post-metamorphic stage. Furthermore, the proximal end of the intestine continues to exit from the dorsal side of the posterior end of the stomach (Figs. 17g, 23). These

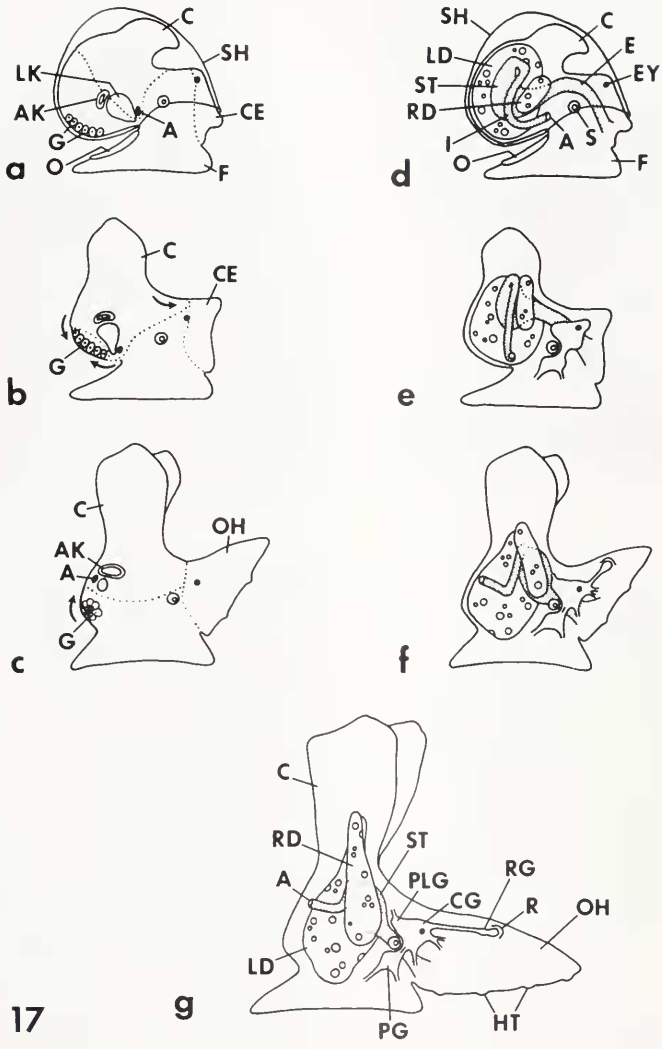


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FIGURE 17. Sketches of successive stages during the metamorphosis of *M. leonina* drawn from reconstructions of serial, one micrometer sections. Figures 17a, 17b, and 17c show post-larva at the time of velum loss, and at 5 and 24 hours after shell loss, respectively. These three diagrams illustrate the migratory movements of the hypertrophied mantle fold and cephalic epithelia (the borders of these epithelia are demarcated by broken lines), the invagination of the gonadal rudiment, and the postero-dorsal displacement of the anus. The arrows indicate specific movements of the mantle fold epithelium. Figures 17d, 17e, 17f, and 17g show post-larvae at velum loss, and at 5, 24, and 48 hours after shell loss, respectively. These four diagrams illustrate the size and positional changes undergone by the component organs of the digestive system during metamorphosis. Abbreviations: A, anus; AK, adult kidney rudiment; C, ceras; CE, hypertrophied cephalic epithelium; CG, cerebral ganglion; E, esophagus; EY, eye; F, foot; G, gonadal rudiment; HT, hood tentacle; I, intestine; LD, left digestive diverticulum; LK, larval kidney complex; O, operculum; OH, oral hood; PG, pedal ganglion; PLG, pleural ganglion; R, rhinophore; RD, right digestive diverticulum; RG, rhinophoral ganglion; S, statocyst; SH, shell; ST, stomach.

observations indicate that the digestive tract of *M. leonina* undergoes partial, but not complete detorsion at metamorphosis.

As shown diagramatically in Figures 17a–c, the larval kidney complex and the

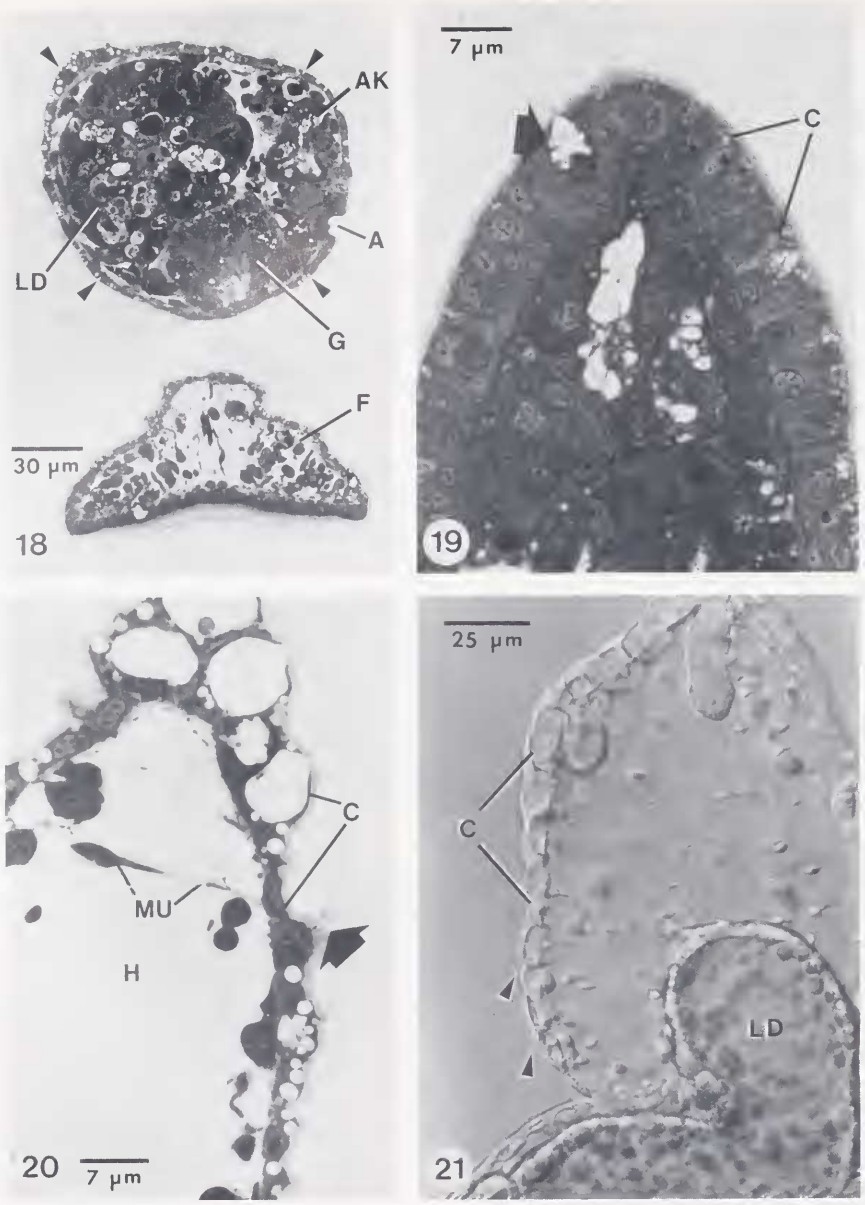


FIGURE 18. Cross section through the posterior portion of the foot (F) and visceral mass at 5 hours after shell loss. The epithelium of the mantle fold (arrowheads) has spread over the visceral mass so as to completely cover the large left digestive diverticulum (LD), the adult kidney (AK), and the invaginated rudiment of the gonad (G). The section also passes through the posterior margin of the anus (A).

FIGURE 19. Section through a primary ceras of a late stage larva of *M. leonina*. Occasional unicellular mucous glands (arrow) are embedded in the pseudostratified columnar epithelium of the ceratal (mantle fold) epithelium (C). The interior of the structure is packed with cells, some of which contain prominent vacuoles.

FIGURE 20. Section through the apical portion of a primary ceras at 5 hours after shell loss. The ceratal epithelium (C) is composed of highly vacuolated, squamous cells and occasional ciliated sensory cells (arrow). A transverse muscle fiber (MU) traverses the expanded hemocoel (H) of the ceras.

FIGURE 21. Photomicrograph using Nomarski differential interference optics of a primary ceras of *M. leonina* during metamorphosis showing the extension of the left digestive diverticulum (LD) into the ceratal hemocoel and patches of stiff cilia (arrows) arising from the ceratal epidermis (C). The photomicrograph indicates that the large vacuoles within the ceratal epidermis are not fixation artifacts.

rudiment of the adult kidney move posteriorly with the anus and distal end of the intestine. The larval kidney complex subsequently degenerates within the post-larval body, whereas the cells of the adult kidney rudiment begin to proliferate and the internal lumen enlarges (Fig. 18).

The diagrams shown in Figures 17d–g illustrate the positional changes exhibited by the organs of the larval digestive system during metamorphosis of *M. leonina.* Unlike the process of gut metamorphosis in the dorid nudibranch *Doridella steinbergae* (Bickell *et al.,* 1981), the stomach of *M. leonina* does not undergo additional torsional displacement at metamorphosis, nor does it shift to the mid-dorsal surface of the large left digestive diverticulum. Although the left digestive diverticulum continues to reside beside the stomach, a dramatic enlargement of the right digestive diverticulum gradually displaces the stomach to a central position within the visceral mass (Figs. 17g, 22). Soon after shell loss, both the left and right digestive diverticula begin to extend into the expanded hemocoel of their respective ceras (Figs. 21, 22).

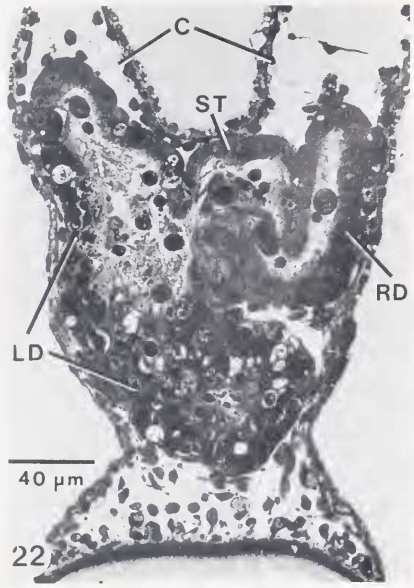
The conversion of the phytoplanktotrophic larva to the carnivorous juvenile—adult necessitates extensive changes of the tissues comprising the larval gut. The cells of the densely ciliated band (style sac) have completely dissociated by the time the post-larva has lost the shell and the gastric shield subsequently peels away from its underlying cells (Fig. 24). Soon thereafter, the cells that produced the larval gastric shield and the cells of the vestibule begin to produce the cuticular material that lines the stomach of the post-metamorphic animal (Agersborg, 1923b) (Fig. 25).

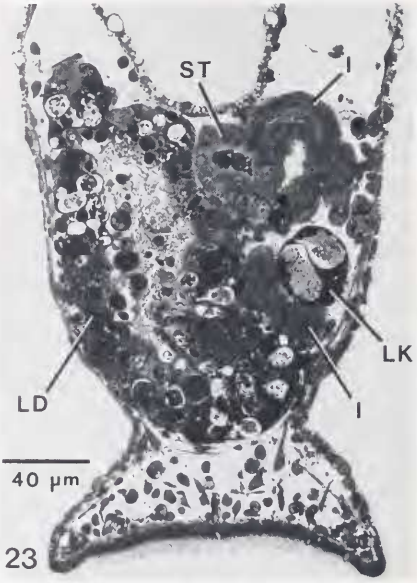
As previously stated, the enlargement of the oral hood is accompanied by the same types of events that occur during expansion of the primary cerata. The hypertrophied cells of the cephalic epithelium convert from a columnar to squamous shape, numerous intracellular vesicles appear, and the enclosed hemocoel becomes inflated. The initial hood tentacles appear as 8 small papillae distributed around the periphery of the hood (Fig. 16). In living animals, particularly after the onset of feeding, prominent nerve tracts extend from the cerebral ganglia to a small cluster of cells underlying each of the hood tentacles (Figs. 26, 27). Transmission electron microscopy has confirmed that these tracts are nerves rather than muscle bundles (Bickell, unpublished observations). The epithelium of each hood tentacle gives rise to several tufts of stiff cilia (Fig. 27) and additional ciliary tufts appear on the ventral surface of the hood during metamorphosis.

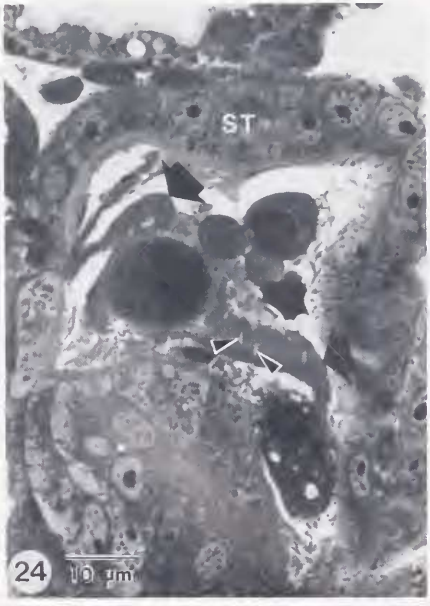
Differentiation of muscles within the periphery of the oral hood enables it to close (compare Figs. 26 and 28) if a tactile stimulus is applied to the ventral surface of the hood. *Melibe leonina* is able to capture and ingest ciliates using the oral hood and oral lips at approximately 2.5 days after shell loss.

Several morphological changes in the nervous system of *M. leonina* can be resolved in one micrometer sections of metamorphic stages. The parapedal commissure can be resolved at 10 hours after shell loss as a slender tract just posterior to the pedal commissure. The cerebrobuccal connectives also become distinguishable at this time and the pleuropedal connectives become distinct from the cerebropedal connectives. By 24 hours after shell loss, a lengthening of the pedal and parapedal commissures and of the cerebrobuccal connectives has occurred. The neuropile region of all the central ganglia enlarges during metamorphosis.

During the period of velum dissociation, a neuronal soma located medio-dorsally within the right pleural ganglion, at the level of the pleuropedal connective, becomes notably larger (10 μm diameter) than the surrounding ganglionic cell bodies (3 to 5 μm diameter) (Figs. 29, 30). By virtue of its size, position, and large nucleus containing a prominent nucleolus, this neuron can be re-identified in all subsequent metamorphic stages. Several other neuronal somata within the right and left pleural







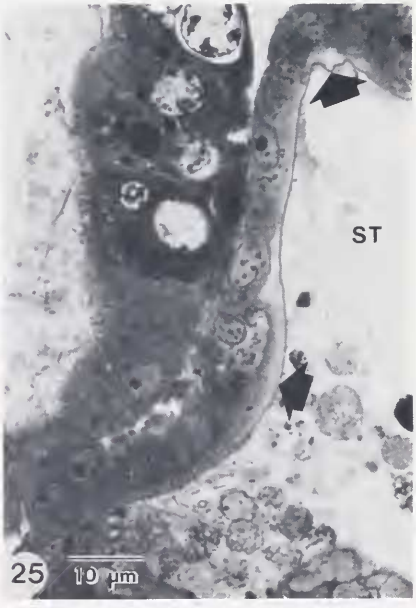


FIGURE 22. Cross section of *M. leonina* at 5 hours after shell loss that passes through the left and right digestive diverticula (LD and RD, respectively) where they enter the stomach (ST). Both diverticula are beginning to project into their respective ceras (C).

FIGURE 23. Cross section of *M. leonina* at 5 hours after shell loss showing the emergence of the intestine (I) from the dorsal side of the posterior end of the stomach (ST). The left digestive diverticulum (LD) and degenerating larval kidney complex (LK) are also shown.

FIGURE 24. High magnification of the stomach area of Figure 22. The larval gastric shield (large arrows), which can be recognized by the presence of small hyaline rods (small arrowheads) embedded in the shield matrix, is sloughing into the lumen of the stomach (ST).

FIGURE 25. High magnification of the wall of the stomach (ST) at 24 hours after shell loss. The arrowheads indicate the cuticle that lines the inner side of the gastric epithelium in post-metamorphic animals.

ganglia become notably larger than their neighbors during the period of metamorphosis.

At the time of shell loss, the rhinophoral ganglia are closely apposed to the antero-dorsal surface of their respective cerebral ganglion and the cells of the cephalic epithelium that directly overlie each of the rhinophoral ganglia are taller and more lightly staining than the surrounding epithelial cells. These patches of thickened epithelium, the presumptive rhinophores, and their associated rhinophoral ganglia are carried anteriorly as the cephalic epithelium expands to form the oral hood. As each rhinophoral ganglion moves away from its ipsilateral cerebral ganglion, the two remain connected by a thick rhinophoral nerve (Fig. 31). The epithelial cells of the presumptive rhinophores proliferate so as to form prominent bulges on the dorsal surface of the enlarging oral hood. Cells bearing tufts of stiff cilia differentiate within the rhinophoral epithelium by 5 hours after shell loss and patches of motile cilia appear during the following 2 days.

Discussion

Although the developmental events that occur during the larval stage of opisthobranchs are similar in kind and sequence, various differences often occur between species. In some cases, these differences can be interpreted as ontogenic anticipation of unique structural features of the post-metamorphic stage or special features to facilitate the success of settlement and metamorphosis or the survival of young juveniles in the adult habitat (Chia and Koss, 1978; 1982; Switzer-Dunlap, 1978; Bickell and Chia, 1979). This phenomenon is illustrated by three unusual features of the late stage larva of *Melibe leonina*. These are: the absence of radular teeth, the appearance of presumptive oral hood tissue, and the precocious development of the primary cerata.

The almost complete omission of the radula—odontophore complex from the sequence of developmental events in *M. leonina* eliminates an unnecessary energy expenditure as this structure has no larval function and is not required for food capture or ingestion in the adult. However, the small size of newly metamorphosed nudibranchs may preclude feeding on the same type of prey or in the same manner as the adults of their species. At least one species of nudibranch utilizes its radula to graze on an organic surface film until sufficiently large to exploit the preferred prey of the adult stage (Perron and Turner, 1977). Juveniles of *M. leonina* cannot employ this type of interim feeding due to the lack of a radula. Instead, metamorphosis in *M. leonina* involves a rapid differentiation of the oral hood, thereby permitting young juveniles to capture small prey in a manner similar to that employed by the adult. Selective pressures acting to promote the rapid formation of the oral hood during metamorphosis may have resulted in the preliminary development of this structure during the final part of the larval stage. Furthermore, non-specific metamorphic induction and the active nature of the juvenile prey (*e.g.*, ciliates) confront newly metamorphosed *M. leonina* with the problems inherent in feeding on organisms having a patchy distribution in time and space. This challenge may have resulted in selection for the greater activity and tactile—positional awareness observed in newly metamorphosed juveniles of *M. leonina*. In response to ciliates colliding with various parts of their body, the juveniles can rapidly turn the anterior body, expand the oral hood, and make a directed and effective capture of the organism. The active prey searching behavior of young *M. leonina* juveniles and their high degree of responsiveness to tactile environmental stimuli contrasts with the behavior of recently metamorphosed juveniles of other opisthobranchs, which tend

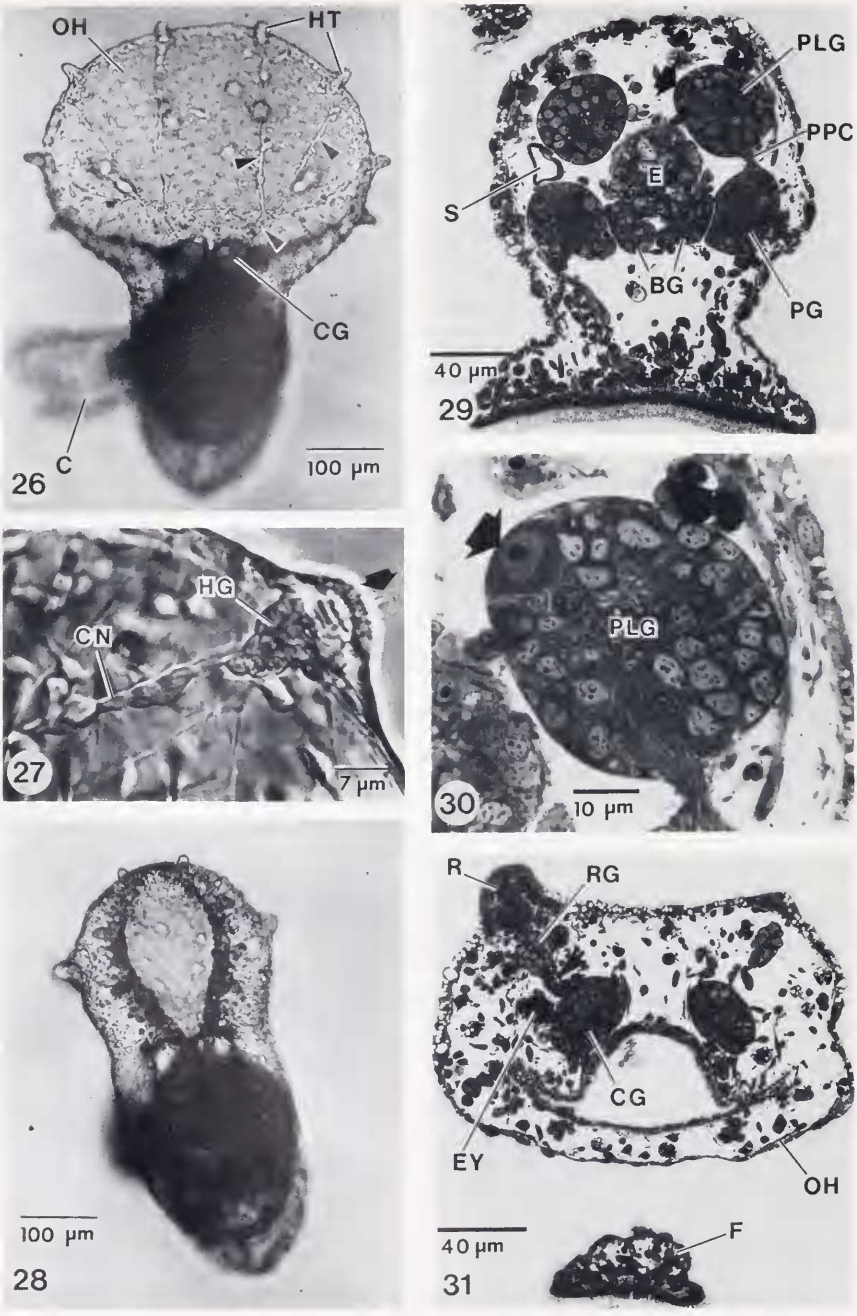


FIGURE 26. Ventral view of *M. leonina* at 5 days after shell loss showing the extended oral hood (OH). Nerve tracts (arrowheads) extend from the cerebral ganglia (CG) to the buds of the peripheral hood tentacles (HT). A ceras (C) is also visible.

FIGURE 27. Photomicrograph using Nomarski differential interference optics of a portion of the oral hood margin showing the terminal region of a cerebral nerve (CN) extending to a peripheral hood ganglion (HG) that underlies a hood tentacle bud. A tuft of stiff cilia (arrow) extends from the epithelium of the tentacle bud.

to be sluggish grazers on the prey organism that induced the metamorphosis of the preceding larval stage (Thompson, 1958; 1962; 1964; Tardy, 1970; Bonar and Hadfield, 1974; Kempf and Willows, 1977; Switzer-Dunlap and Hadfield, 1977; Chia and Koss, 1978; Bickell, 1978; and others).

The hypertrophy of the larval cephalic epidermis has not been noted in premetamorphic veligers of dorid nudibranchs, which tend to lack a large oral veil over the mouth, but is shown in drawings by Thompson (1962) of premetamorphic veligers of *Tritonia hombergi* (Dendronotacea) and by Tardy (1970) of *Aeolidiella alderi* (Aeolidacea). The juveniles and adults of both these species have a prominent oral veil that is derived from this hypertrophied cephalic tissue. These observations confirm that the oral hood of *M. leonina* and the oral veil of other nudibranchs are homologous structures.

The appearance of ceratal rudiments in the larval stage of nudibranchs has not been reported previously, although many aeolids and dendronotids have been reared in the laboratory. It has been suggested that the thin-walled cerata of nudibranchs provide an increased surface area for gas exchange with the environment (see Morton, 1958). This hypothesis is strengthened by the fact that the metabolically active digestive diverticula often extend into the cerata. Ajeska and Nybakken (1976) found that oxygen consumption/gm body weight was an inverse function of animal size in *M. leonina*. They suggested that the higher metabolic rate of young juveniles reflects the fact that they must actively seek-out their benthic prey, whereas larger animals simply extend their hood into the surrounding waters to intercept passing zooplankton. Of the 10 species of newly metamorphosed opisthobranchs that the present authors have observed, *M. leonina* young juveniles are most active. Together, these observations suggest that the development of ceratal rudiments during the larval stage of *M. leonina* and their rapid expansion and invasion by the digestive diverticula during metamorphosis may be necessary to sustain a high oxygen demand resulting from an active juvenile life style.

The present study of larval development and metamorphosis of *M. leonina* provides the second histological description of gut metamorphosis in a planktotrophic nudibranch veliger. As in the dorid nudibranch, *Doridella steinbergae* (Bickell *et al.*, 1981), the morphologically complex stomach of *M. leonina* veligers is transformed to the post-metamorphic stomach by dissociation of the cells comprising the ciliated band (style sac) and loss of the gastric shield. In *Doridella steinbergae*, Bickell *et al.* (1981) speculated that the gastric shield was lost by dissociation of the underlying cells. Observations made in the present study indicate that the gastric shield is simply sloughed from the gut wall; the underlying cells are retained and subsequently secrete a portion of the cuticle that lines the stomach of the post-metamorphic stage.

FIGURE 28. Same animal as that in Figure 26 showing closure of the oral hood by contraction of muscles extending along the hood periphery.

FIGURE 29. Slightly oblique cross section through the esophageal region (E) of *M. leonina* at 24 hours after shell loss. Note the left statocyst (S), buccal ganglia (BG), pleural ganglia (PLG), and pedal ganglia (PG). The arrowhead indicates the distinctive neuronal soma (see Fig. 30) that is situated dorsomedially within the right pleural ganglion at the level of the pleuro-pedal connective (PPC).

FIGURE 30. Enlargement of the pleural ganglion (PLG) from Figure 29 indicating the large neuronal soma (arrow) containing a prominent nucleolus.

FIGURE 31. Slightly oblique cross section through the base of the oral hood (OH) and the anterior end of the foot (F) at 24 hours after shell loss showing the developing rhinophore (R) and its underlying rhinophoral ganglion (RG) on the left side. A rhinophoral nerve (arrowhead) extends between the rhinophoral ganglion and the cerebral ganglion (CG). The section also passes through the left eyespot (EY).

The stomach of *M. leonina* does not undergo additional torsional displacement during metamorphosis, as observed in *D. steinbergae* (Bickell *et al.,* 1981), nor does it exhibit complete detorsion, as described for the aeolid nudibranch *Phestilla sibogae* (Bonar and Hadfield, 1974). In *M. leonina,* the dorso-lateral position of the anus and the fact that the intestine emerges from the dorsal aspect of the stomach are post-metamorphic vestiges of the torted larval digestive tract.

As is typical of most opisthobranch veligers, those of *M. leonina* possess a large left and a much smaller right digestive diverticulum. The few histological investigations that have considered gut metamorphosis in opisthobranch larvae indicate that the right diverticulum 'disappears' at or soon after metamorphosis in the dorids *Adalaria proxima* (Thompson, 1958) and *Doridella steinbergae* (Bickell *et al.,* 1981). Thompson (1962) reported the persistence of this organ for a period of time after metamorphosis in the dendronotid *Tritonia hombergi* but noted that it eventually became impossible to differentiate the right diverticulum from the left. Nevertheless, on the basis of adult morphology, the right diverticulum appears to persist in the Dendronotacea, Arminacea, and Aeolidacea (see Hyman, 1967, p. 443). Our study of morphogenesis in larvae and juveniles of *M. leonina* shows that both the right and left diverticula are retained during metamorphosis. Each diverticulum proliferates into its ipsilateral ceras and opens separately into the stomach. This feature persists into the adult stage, although the main duct of the left digestive diverticulum, but not the right, eventually branches at its point of exit from the stomach (Agersborg, 1923b).

Larval settlement and metamorphosis has been observed in three species of dendronotid nudibranchs. *Tritonia hombergi* is typical of many opisthobranchs (see Hadfield, 1978) in that metamorphosis will occur only in the presence of its post-metamorphic prey, *Alcyonium digitatum* (Thompson, 1962). Metamorphosis of the larvae of *Tritonia diomedia* is promoted by the preferred pennatulacean prey of the adults, but metamorphosis will also occur without this external inducer. Kempf and Willows (1977) suggested that the absence of absolute dependence on an external metamorphic trigger in *T. diomedia* may relate to the fact that adults will also feed on several other pennatulaceans. In *M. leonina,* the presence of a substratum appears to be the only requirement for the onset of larval settlement and metamorphosis. The prey of young juveniles (which was benthic ciliates and crustacean nauplii in this study and benthic crustaceans and bivalve spat in the field study of Ajeska and Nybakken, 1976) probably occurs ubiquitously on marine substrates, and the zooplanktonic prey of larger juveniles and adults is continuously transported through coastal waters. Therefore, the need for specific metamorphic induction to ensure a benthic food source (Thompson, 1964) seems unnecessary in this species.

Despite the apparent absence of environmental induction of metamorphosis, populations of *M. leonina* are consistently found in eel grass and kelp beds located in protected waters (Agersborg, 1923a; Hurst, 1968; Ajeska and Nybakken, 1976). Pelagic individuals of this species, which include the larvae and post-metamorphic animals that have become dislodged from a surface (Hurst, 1968), may become passively concentrated in areas of reduced water flow. The buoyant fronds of eel grass and certain large kelp species that are typical of these locations might be expected to promote the survival of *M. leonina* because the plants provide a submerged, tidal adjusting attachment substratum (*M. leonina* cannot withstand atmospheric exposure) that is suspended within the upper levels of the water column where the flow of plankton-carrying currents is greatest.

Melibe leonina offers considerable potential for studies on opisthobranch neurodevelopment. Unlike many other species, reproductive adults and egg masses can

be collected throughout the year (Hurst, 1967). Furthermore, the successful rearing of juveniles on ciliates followed by commercially available *Artemia* nauplii simplifies the problem of obtaining a continuous supply of food for the post-metamorphic stage. The central ganglia of *M. leonina* include many large, identifiable neurons (Hurst, 1968) and the present study has shown that several neuronal cell bodies become morphologically distinct during metamorphosis. Finally, the rapid formation of cerebral nerve tracts innervating the oral hood and their visibility through the transparent epithelium of this structure may allow investigation of axonal guidance during neurodifferentiation.

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